The Effect of Vitamin B_{12} on the Anemia and Combined System Disease of Addisonian Pernicious Anemia

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SUMMARY

The effect of the parenteral administration of vitamin B_{12} has been observed in eight patients with Addisonian pernicious anemia.

Vitamin B_{12} in initial doses of 50 micrograms or 25 micrograms induced satisfactory reticulocyte response and a return of erythrocyte count to within normal range in 60 days.

In only two of the patients were secondary reticulocyte responses induced on a second injection of vitamin B_{12}.

Concurrently with the hemopoietic response, the bone marrow changed from megaloblastic hyperplasia to normoblastic distribution.

The paresthesias associated with combined system disease as well as disturbances in position sense and locomotor function may be entirely relieved or greatly diminished following injections of vitamin B_{12}.

Maintenance injections of vitamin B_{12} may be from 30 to 50 micrograms at intervals of one month, the amount depending upon the individual case.

Vitamin B_{12} may be used without untoward symptoms in patients previously sensitive to liver extract.

IN 1926 it was demonstrated by Minot and Murphy[5] that whole raw liver was effective in the treatment of Addisonian pernicious anemia. Soon after this, a search was begun by investigators to determine the factor in liver responsible for its effects on the hematopoietic and neurologic systems. Cohn[3] in 1927 produced an extract of liver called "Fraction G" which became available for oral use. This was a water soluble material obtained from protein precipitation. The early preparations contained histamine-like substances which limited their parenteral use in patients because of their blood pressure reducing effect. In 1930 a more highly purified product was produced which was first called crude liver extract and contained one or two units per cc. of extract.[6] Within the past ten years, with the development of more highly refined techniques, such as salting-out processes, and reducing extracts to temperatures of an extremely low degree, a product of higher concentration has been produced containing 10 to 15 units per cc. of extract.[7]

The chemical formula of liver extract is not known. West and others, after many years of study, concluded that it was probably a polypeptide. Irrespective of this paucity of knowledge concerning its chemical structure, there is evidence suggestive of a close relationship between liver extract and the vitamin B complex. Wills,[14,15] Wintrobe,[16] and others have shown that in certain macrocytic anemias, large doses of yeast, a substance rich in vitamin B components, will induce a reticulocyte response followed by an increase in the number of erythrocytes in the circulating blood. Studies undertaken in our laboratory have shown comparable results. However, with the isolation and synthesis of each new member of the complex their ineffectiveness in the treatment of pernicious anemia have been established.[2] It is to be observed in Figure 1 that no increase in production of blood occurred after the administration of riboflavin, nicotinic acid, or synthetic vitamin B_6, but that a response occurred when the patient was fed 45 gm. of yeast daily.

The isolation and synthesis of folic acid (Pteroylglutamic acid), a result of the cooperative efforts of Dr. Yellapragada Subbarow, other investigators and the research facilities of the Lederle Laboratories, furnished, for the first time, a pure substance which could apparently produce a hematologic and clinical remission in Addisonian anemia. Although hypotheses have been developed as to the probable physiologic mechanism through which folic acid works, its exact role in hematopoiesis is still a mystery.

One of the reasons for failure to advance knowledge concerning the identity or specificity of liver extracts and related substances has been the lack of any chemical or biological test which might serve to determine the potency of the fractions being investigated. This has been accomplished only by administering material to patients with Addisonian pernicious anemia who are in relapse. Experimental data is now accumulating to show that a bacterium may be used to supply this deficiency. In 1948 Shorb[9] reported the finding that Lactobacillus lactis Dorner, when cultivated in a suitable basal medium, grew when refined liver extract was added to the menstruum. When assays of various commercial liver extracts were carried out by determining the minimum quantity that would support growth, Shorb found an almost linear relationship to the unit potency of the extracts. Recently, Rickes[8] and his associates have isolated a crystalline substance from liver extract in the form of small red needles and Shorb,[10] who assayed its potency value for L. lactis

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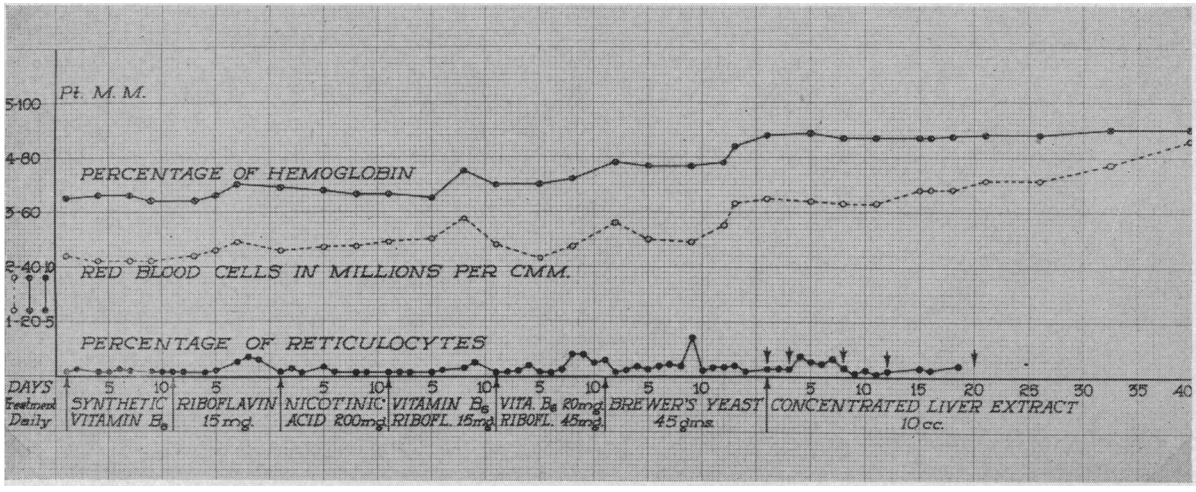

Figure 1.—A case of Addisonian pernicious anemia. Components of the vitamin B complex given by mouth are without hematopoietic effect. There was satisfactory response to 45 gm. daily doses of yeast.

Dorner, found that 0.000013 micrograms per ml. was sufficient to produce half maximal growth of L. lactis in a 23-hour growth period. With respect to weight it was 11,000 times as potent as an arbitrarily selected standard liver extract. This substance has been designated as vitamin B_{12}. West,[13] in 1948, reported that vitamin B_{12} is of great value in the treatment of Addisonian pernicious anemia. Further reports substantiating the effectiveness of vitamin B_{12} in the treatment of pernicious anemia have been published by Spies[11, 12] and Castle.[2]

During the past year on the wards and in the Out-Patient Department of the University of California Medical School, we have had an opportunity to observe the effects of vitamin B_{12} on the anemia and neurological abnormalities of a group of patients with Addisonian pernicious anemia. The case histories including the pertinent physical findings and laboratory data are reported.

GROUP I

This group consists of four cases of uncomplicated pernicious anemia.

CASE REPORTS

CASE 1: A 70-year-old white male was admitted to the medical service of the Out-Patient Department on May 24, 1948. He complained of progressive weakness, pallor, anorexia, and sore tongue of three months' duration. During the preceding four weeks he had become aware of some exertional dyspnea and a desire to sleep a great deal. His appetite was poor and he was frequently nauseated. Friends commented that his skin appeared pale and yellowish. He denied consciousness of numbness and tingling in the fingers or toes, or of instability of gait. There was no known family history of anemia.

On physical examination, the patient appeared slightly obese, and there was a pale yellowish discoloration of the skin. The sclerae was faintly icteric. The tongue was pale and smooth. No other positive findings were elicited. Examination of the central nervous system was also negative.

Erythrocytes numbered 1,370,000 per cu. mm. with a hemoglobin of 6.8 gm. per 100 cc. (47 per cent), and the leukocyte count was 4,600 per cu. mm. The packed cell volume was 20 cc. per 100 cc. of blood. The mean corpuscular volume was 145 cubic microns; mean corpuscular hemoglobin concentration 34 per cent; and the mean corpuscular hemoglobin 49 micro micrograms. The color index was 1.7. The reticulocytes were 0.6 per cent. An analysis of the gastric secretion revealed an absence of free hydrochloric acid and pepsin which persisted following the subcutaneous injection of histamine. Specimens of bone marrow removed by aspiration of the sternal cavity showed marked megaloblastic and erythroblastic hyperplasia.

On the sixth day following examination, treatment with vitamin B_{12} was started by the intramuscular injection of 25 micrograms.* The reticulocytes began to rise on the third day following treatment and reached a peak of 11.6 per cent on the seventh day. At the onset of the reticulocyte rise the patient expressed a feeling of improvement and a return of appetite. On the 16th day following treatment, the erythrocyte count had risen to 2,990,000 per cu. mm. and the hemoglobin to 10.1 gm. per 100 cc. (70 per cent). The reticulocytes returned to a pre-treatment level of 1.6 per cent. Examination of aspirated sternal marrow showed a normal pattern. On this day the patient was given a second injection of 25 micrograms of vitamin B_{12} to determine whether or not a secondary reticulocyte rise could be induced. It is to be observed that there was a rise in the reticulocytes from 0.6 per cent to a peak of 5.2 per cent. Following this the reticulocytes returned to a low level of 0.4 per cent.

Twelve weeks following the initial treatment the erythrocytes numbered 4,100,000 and the hemoglobin content was 12.7 gm. per 100 cc. (92 per cent).

Although the need for continued treatment was emphasized to the patient, he did not return to the clinic for a period of three months. On December 10 he reported to the clinic because of a return of weakness. Erythrocytes numbered 1,580,000 and the hemoglobin was 8 gm. per 100 cc. (57 per cent). Treatment was reinstituted with an injection of 50 micrograms of vitamin B_{12}. There was a repetition of the reticulocyte response and a peak of 13 per cent was recorded on the sixth day. Four weeks following the injection of vitamin B_{12} erythrocytes numbered 3,500,000 and the hemoglobin was 10.8 gm. per 100 cc. (75 per cent). On February 11, 1949 (eighth week) erythrocytes were 4,160,000 and the hemoglobin 14.5 gm. per 100 cc. The data

*Supplied by Dr. Augustus Gibson of Merck & Co., Rahway, New Jersey.

showing this patient's response to vitamin B_{12} appears in Figures 2, 3, and 4.

CASE 2: A white female, aged 28 years, was first observed in the Out-Patient Department in 1933 with a complaint of weakness, palpitation, and dyspnea. There were no paresthesias. Aside from obesity, the physical examination showed no symptoms or signs of clinical significance. There was no clinical evidence of disease of the neurological system. Laboratory studies revealed an erythrocyte count of 2,000,000 per cu. mm.; hemoglobin content 54 per cent of normal (7.5 gm. per 100 cc., Sahli). Achylia gastrica persisted despite the parenteral injection of histamine. The halometer reading (average diameter of the erythrocyte) was 8.3 microns; the saturation index was 1.31. Accordingly, a diagnosis of hypochromic macrocytic anemia was made. The patient responded well to parenteral injections of liver extract. On the 25th day following the institution of therapy, erythrocytes numbered 4,100,000 per cu. mm., and the hemoglobin content was 88 per cent (12.4 gm. per 100 cc. Sahli).

(It is of interest that in 1933 the patient's mother was examined and found to be suffering from hypochromic anemia. The erythrocyte count was 4,170,000 per cu. mm., and the hemoglobin content was 54 per cent (7.5 gm. per 100 cc., Sahli). The mother was reexamined in 1943 at which time she showed the characteristics of Addisonian pernicious anemia).

The patient refused treatment for about three years because of marital difficulties. On October 29, 1948, she was admitted to the Out-Patient Department complaining of weakness and dyspnea on exertion. The erythrocyte count was 1,410,000 per cu. mm. and the hemoglobin 47 per cent (6.8 gm. per 100 cc., Sahli). The mean corpuscular volume was 170 cubic microns; the mean corpuscular hemoglobin 41 micro micrograms; and the mean corpuscular hemoglobin concentration 28 per cent.

The patient was given a parenteral injection of 50 micrograms of vitamin B_{12}. Figure 5 shows response to therapy. It is to be noted that within 24 hours the reticulocytes had risen from 0.8 per cent to 7.6 per cent, and reached a peak of 8.8 per cent in 120 hours (fifth day). The patient was given another injection of 50 micrograms of vitamin B_{12} to determine whether or not a secondary reticulocyte rise could be stimulated. However, this did not occur. Erythrocyte count and hemoglobin attained normal levels in 60 days.

CASE 3: A white male, aged 66 years, reported to the Out-Patient Department on January 6, 1947, complaining of epigastric distress. He stated that he had felt well until seven or eight weeks previously when he first noticed a vague soreness in the epigastrium accompanied by nausea and some gaseous eructation. His weight had averaged 205 pounds until two months previously; at the time of examination it was 192 pounds. The patient was a normally developed, moderately obese man, in no apparent distress. The positive physical findings consisted of a moderate degree of

PERNICIOUS ANEMIA NO I

J.E.R. Age. 70 O.P.D. 48690 U.No. 154124.

DATE	R.B.C.	Hgb % gms.	Ret	Platelets	W.B.C.	
6-1-48	1.37	47-6.8		340,000	4600	0.2 cc vit. B_{12} given i.m. 0.025 mg/cc
6-2-48	1.48	46-6.6	0.6			0.2 cc vit B_{12} given i.m
6-3-48			2.2			
6-7-48	1.77	54-7.8	11.6			
6-8-48			8.6			
6-9-48	2.31	56-8.1	8.6			
6-10-48			6.4			
6-11-48	2.03	56-8.1	7.2			
6-12-48			3.8			
6-14-48	2.50	64-9.2	4.4	385,000		
6-15-48			1.2			
6-16-48	2.99	70-10.1	1.8			

Figure 2.—*Case 1.* Response of reticulocytes and course of blood counts on parenteral injection of 25 micrograms of vitamin B_{12}.

PERNICIOUS ANEMIA NO II.

J.E.R. Age 70. O.P.D. 48690 U.NO. 154124.

DATE	R.B.C.	Hgb. % gms.	Ret.	Platelets	W.B.C.	
7-16-48	2.87	74-10.7				B_{12}-10cc. i.m. 0.025 mg. m.
7-19-48	2.70	71-10.2	0.6			
7-20-48			3.4			
7-21-48	2.64	77-11.1	4.6			
7-22-48			4.6			
7-23-48	3.39	84-12.1	5.2			
7-28-48	3.07	84-12.1	1.0			
7-29-48			0.4			
8-5-48	3.43	86-12.4		200,000		
8-12-48	3.62	90-13.04		225,000		
8-20-48	3.87	88-12.7				
8-27-48	4.10	92-12.7		380,000		
9-10-48	4.28	84-12.1				

Figure 3.—*Case 1.* Secondary reticulocyte response induced by second injection of 25 micrograms of vitamin B_{12}. Return of red blood cells and hemoglobin within normal range in 60 days.

PERNICIOUS ANEMIA

NAME: J.E.R. AGE: 70 (Re-entry)

DATE	R.B.C.	Hgb. % Gms.	RET	W.B.C.	TREATMENT
12-10-48	1.58	57			Vit. B_{12} .050 mgm. given I.M.
12-13-48	1.91	61	6.0		
12-14-48			10.8		
12-15-48	1.89	60	8.8		
12-16-48			13.0		
12-17-48	2.41	65-9.4	8.4		
12-19-48	2.43	68-9.8			Vit. B_{12} .020 mgm. given I.M.
1-7-49	3.12	79-11.4			
1-10-49	3.50	75-10.8			Vit. B_{12} .050 mgm. given I.M.
1-14-49	3.34	86-12.4		7600	
1-21-49	3.43	85-12.3		7500	.050 mgm. B_{12} given I.M.
1-28-49	4.13	85-12.3		11,050	
2-4-49	4.08	88-12.7		12,650	
2-11-49	4.00	106-15.3			

Figure 4.—*Case 1.* Shows recurrence of anemia four months after last injection of vitamin B_{12} but satisfactory response of blood on further administration of vitamin B_{12}.

PERNICIOUS ANEMIA

(IN RELAPSE)

NAME: E. G. AGE: 43

DATE	R.B.C.	Hgb. % Gms.	RET %	W.B.C.	TREATMENT
10-29-48	1.41	47-6.8	0.8	4500	Vit B_{12} .050 mgm. given I.M.
11-1-48			7.6		
-2-			6.8		
-3-	1.73	57-8.2	8.8	7900	
-4-	2.15	58-8.4	7.0		
-5-	2.28	58-8.4	6.0		
-8-	2.68	72-10.4	6.0		
-9-			0.5		
-10-	2.84	81-11.7	2.6		
-11-			2.6		
-12-	2.91	83-12.0	1.6		Vit B_{12} .050 mgm. given I.M.
-15-	3.14	84-12.1	1.6		
-16-			0.8		
-17-	3.04	87-12.6	2.2		
-18-			1.2		
-19-	3.12	82-11.8	1.4		
12-31-48	4.37	94-12.9			Vit B_{12} .030 mgm. given I.M.

Figure 5.—*Case 2.* Response of reticulocytes and blood counts to 50 micrograms of vitamin B_{12}. No response of reticulocytes occurred following second injection.

PERNICIOUS ANEMIA

NAME: MfC R.M. AGE: 67

DATE	R.B.C.	Hgb % Gms	RET	W.B.C.	TREATMENT
10-6-48	1.01	35-50	1.8	3500	Megaloblastic bone marrow
-9-			3.0		
-10-			2.6		Vit B_{12} .025 mgm. given I.M.
-11-	1.16	44-63	15.2		
-12-			16.2		
-13-	1.60	45-65	19.4		
-14-			16.2		
-15-	2.06	51-73	10.8	5700	
-16-			4.4		
-18-	2.49	61-87	3.2	9250	
-22-	2.59	61-87			
-29-	2.31	64-92	0.2		Vit B_{12} .025 mgm. given I.M.
11-1-48	2.84	63-91	0.2		
-4-	3.31	64-92	1.0		
-8-	3.45	80-116	1.2		
12-3-48	3.26	86-124			Patient left clinic

Figure 6.—*Case 3.* An injection of 25 micrograms of B_{12} resulted in an erythropoietic response. Patient left clinic before course of observation completed.

Diagnosis: Pernicious Anemia

Name: Mrs. J. P. Age 47

DATE	RBC	Hgb. % Gms.	Ret. %	WBC	TREATMENT
10 8 48	1.64	42-7.1		3400	←25 Micrograms
9			5.6		B_{12} given
11			7.2		
12			15.		
13			15.8		
14			20.8		
15			11.0		
16			7.6		
18			7.0		
19			4.2		
20	2.75	59-10.0	4.0	4050	
22					←25 Micrograms
25			1.6		B_{12} given
27	3.28	69-11.6	2.2	4750	
11-12-48	3.70	74-12.5		6200	
12-3-48	3.43	10.4		4850	
17	5.33	19.1		10,600	

Figure 7.—*Case 4.* Satisfactory hematopoietic response to 25 micrograms of vitamin B_{12}.

generalized arteriosclerosis associated with a systolic blood pressure of 165 mm. of mercury and a diastolic pressure of 76 mm. The erythrocyte count was 4,660,000 per cu. mm., leukocytes numbered 10,360, and the hemoglobin content was 100 per cent of normal (14.5 gm. per 100 cc., Sahli). Gastric analysis showed a persistent achlorhydria despite the parenteral injection of histamine. Stools were negative for occult blood. Roentgenographic studies revealed no abnormalities in the gastrointestinal tract or gall bladder.

The patient did not return to the Out-Patient Department until October 4, 1948, when he complained of progressively increasing dyspnea of four months' duration, anorexia, and a loss of 35 pounds in weight. His family had observed that his skin had become pale and sallow. There had been no numbness or tingling in the fingers or toes, or soreness of the tongue.

Erythrocytes numbered 1,000,000 per cu. mm.; hemoglobin was 35 per cent (5 gm. per 100 cc.); leukocytes numbered 3,500, and the platelets 150,000 per cubic millimeter. The icterus index was 10 units and the volume of packed red blood cells was 15 cc. per 100 cc. of blood. The mean corpuscular volume was 148 cubic microns; mean corpuscular hemoglobin concentration 33 per cent; and the mean corpuscular hemoglobin 40 micro micrograms. A specimen of sternal marrow was aspirated and examination showed the presence of large numbers of megaloblasts consistent with the diagnosis of pernicious anemia. On October 8, 1948, the patient was given an intramuscular injection of 25 micrograms of vitamin B_{12}. The subsequent reticulocyte response and rise in erythrocytes are recorded in Figure 6. At the end of 48 hours, the reticulocytes had increased from 3 per cent to 15 per cent and reached a peak of 19 per cent at 96 hours. A secondary reticulocyte response did not appear following a second injection of liver extract. Just prior to the end of 60 days of observation, erythrocytes numbered 3,280,000 and the hemoglobin was 85 per cent of normal. At this time it became necessary for the patient to travel some distance so that further counts were not obtainable.

CASE 4: A white, married female, aged 47, was first examined on September 12, 1948, at which time she complained of headache, weakness, loss of weight, swelling of ankles, and palpitation. These symptoms had been getting progressively worse over a period of about four months.

On physical examination it was noted that there was a lemon-yellow color to the skin and pallor of the mucous membrane. The tongue appeared smooth. A murmur, systolic in time, was heard in the region of the apex in the heart. The spleen was not enlarged. Erythrocytes numbered 1,640,000 per cu. mm. and the hemoglobin content was 7.1 gm. per 100 cc. (42 per cent). Leukocytes numbered 3,400 per cubic millimeter. Examination of aspirated sternal marrow showed pronounced megaloblastic hyperplasia. The gastric secretion showed an absence of free achlorhydria.

The patient was given 25 micrograms of vitamin B_{12} intramuscularly. In 48 hours the reticulocytes were 7.2 per cent, and in 96 hours had attained a peak of 20.8 per cent. When the reticulocytes had returned to normal numbers, another injection of 25 micrograms of vitamin B_{12} was given. However, a secondary reticulocyte response did not occur. In about 60 days the erythrocyte count and hemoglobin were within normal limits as recorded in Figure 7.

COMMENT ON THE CASES IN GROUP I

It may be seen on study of the charts of these four patients that they all responded well to doses of 50 micrograms or equally well to 25 micrograms of vitamin B_{12}. When the reticulocyte count had fallen, a secondary response was induced in only one patient following a second injection of 25 micrograms of B_{12}. This indicated that in the patients not showing a secondary response the treatment was optimum and was submaximal in the other patient. Improvement was maintained in the patients with injections of 25 micrograms of B_{12} repeated every two weeks. The erythrocytes had returned to within normal range within 60 days in all patients. The one exception (Case 1) was a patient who voluntarily discontinued treatment. At the end of four months, an examination of his blood showed return of anemia of pronounced degree. A second remission resulted from renewed injections of vitamin B_{12}.

GROUP II

Three cases of pernicious anemia complicated by combined system disease.

CASE REPORTS

CASE 5: A 76-year-old white male of Swedish descent entered the San Francisco Hospital in 1944, at which time a diagnosis of pernicious anemia was made. Erythrocytes numbered 680,000 per cu. mm. and the hemoglobin content was 5 gm. per 100 cc. A remission was induced by injection of suitable amounts of liver extract.

The patient entered the San Francisco Hospital for the second time on October 19, 1948, because of recurrence of anemia. It was learned that anemia had been well controlled in the interim by injections of liver extract every three weeks. Four months prior to the present entry, the patient had a "heart attack" which confined him to bed and prevented him from returning to the clinic for needed liver extract therapy. During the previous few weeks he had noticed the occurrence of numbness and tingling in the tips of the fingers and toes.

On physical examination the patient appeared well nourished and well developed. The skin and sclerae were of a lemon-yellow tint. Dyspnea on exertion was obvious. There was generalized arteriosclerosis. A slight increase in the area of cardiac dullness to the left of the midclavicular line was noted. The radial pulse showed gross irregularity as to rate and volume as well as a pulse deficit. The vibratory sense was diminished at the ankles and toes. No abnormalities of the tendon reflexes were elicited.

Examination of the blood showed erythrocytes numbering 870,000 per cu. mm. and hemoglobin of 4.2 gm. per 100 cc. The color index was 1.7. Analysis of the gastric secretion revealed achlorhydria which persisted after the injection of histamine. Aspirated sternal marrow showed pronounced megaloblastic hyperplasia.

On October 23, 1948, 50 micrograms of vitamin B_{12} was injected parenterally. At 48 hours the reticulocytes had risen from 1.3 per cent to 5.3 per cent and in 96 hours had attained a peak of 32 per cent. Examination of the sternal marrow at this time showed erythroblastic and normoblastic hyperplasia. On the 15th day the reticulocytes had returned to pre-treatment level. Erythrocytes numbered 3,000,000 per cu. mm. and hemoglobin was 7.5 gm. per 100 cc. The bone marrow showed a normal myelogram. By this time the patient's ability to discern the vibrations of the tuning fork had returned and the paresthesias of the fingers had disappeared.

Two weeks after the first injection of vitamin B_{12} the patient received a second injection of 50 micrograms to determine whether or not a second reticulocyte rise could be induced. This did not occur.

Sixty days after treatment was instituted, erythrocytes numbered 385,000 per cu. mm., and the hemoglobin was 89 per cent of normal. The disease was complicated at this time by the occurrence of cardiac decompensation for which it was necessary to hospitalize the patient. Data on this patient is shown in Figure 8.

CASE 6: A white male, aged 65, was admitted to the San Francisco Hospital January 28, 1949, complaining of numbness and tingling in the fingers and toes. He stated that two years before entry he had visited a physician because of the appearance of a yellow hue to the skin. A diagnosis of pernicious anemia was made, and he was given injections of liver extract three times a week for two weeks and then once a week for five months. He did not return for continued therapy because he felt sufficiently improved. Eight months following the cessation of the original therapy, there was exacerbation of symptoms, and the patient again consulted a physician. He received injections of liver extract over a period of eight months when, because of a feeling of well-being, he again discontinued treatment.

Six weeks prior to entering the hospital he noticed numbness and tingling of the tips of fingers and toes. This progressed in severity to the point that for the preceding two weeks he had experienced difficulty in walking. He was uncertain as to the stability of his feet, necessitating the watching of his feet while walking. Also, in the preceding two weeks he found it necessary to spread his feet farther apart in order to maintain balance. He also noticed difficulty in writing and in handling small objects.

PERNICIOUS ANEMIA
(IN RELAPSE)
NAME: Mr. L. AGE: 76

DATE	R.B.C.	Hgb. %Gms.	RET	WBC	TREATMENT
10-23-48	870,000	4.2	1.3		← Vit. B_{12} .050 mgm. given I.M.
-24-			1.9		Bone marrow Megaloblastic and Erythroblastic hyperplasia.
-25-	916,000	4.4	5.3		
-26-			11.0		
-27-	1.52	31-45	32.0		← Bone marrow, Erythroblastic and Normoblastic hyperplasia.
-28-			27.0		
-29-	1.65	40-56	22.2	5200	
-30-			16.0		
-31-	2.05	40-56	11.0		
11-1-48	2.52	41-59	14.6		
2			3.8		
3	2.24	61	11.6		
4	2.33	43-64	8.8		
5	2.42	49-70	7.6		
7	2.62	55-80	4.8		← Vit. B_{12} .050 mgm. I.M.
9	3.04	75	3.8		← Bone marrow Normoblastic.
12	3.00	58-84	1.1		
17	3.17	68-98	0.2		
12-3-48	3.16	84-121			← Bone marrow normal. Vit. B_{12} .050 mgm. I.M. Hospitalized for Cardiac decompensation.
10	3.39	74			

Figure 8.—*Case 5.* Changes from megaloblastic to normoblastic marrow accompany hematopoietic response. Paresthesias due to combined system disease subsided 15 days after injection of 50 micrograms of vitamin B_{12}.

During the preceding two years his weight had dropped from an average of 185 pounds to 155 pounds. There was no history of sore tongue. There was no history of pernicious anemia in the family.

On physical examination the patient appeared older than his stated age. He was underweight, the hair was white, the skin appeared "lemon-yellow," and there was a mild icteric tint to the sclerae. The tongue appeared smooth and was not "beefy-red" in color. Examination of the nervous system revealed hyperactive biceps and patellar tendon reflexes. The Romberg test was designated as postive. The vibratory sense was diminished over the feet.

Erythrocytes numbered 1,400,000 per cu. mm. and hemoglobin was 4.8 gm. per 100 cc. of blood. The mean corpuscular volume was 109 cubic microns, and the mean corpuscular hemoglobin 40 micro micrograms. A bone marrow specimen aspirated from the sternum contained 37 per cent megaloblasts. The patient had histamine-fast achlorhydria.

On January 28, 1949, the patient was given an intramuscular injection of 50 micrograms of vitamin B_{12}. On this occasion the reticulocytes were 0.7 per cent but within 72 hours had risen to 6.4 per cent. In 96 hours they had attained a peak of 24.4 per cent following which they returned to pre-treatment level. On February 18 the patient received an injection of 25 micrograms of vitamin B_{12}. This was followed by a secondary rise in the reticulocytes to 8 per cent.

Seven days after the first injection of vitamin B_{12}, the patient noticed a lessening of the paresthesias in the hands and feet. At the same time, he began to gain in strength, his gait became more stable, the Romberg was recorded as negative, but no change was noted in the tendon reflexes or vibratory sense.

Diagnosis: Pernicious Anemia
Name: Mr.C.B. Age: 65

DATE	RBC	Hgb. % Gms	Ret %	WBC	PMN F-NF	PME	PMB	LL	Mo.	TREATMENT
1-28-49	1.40		0.7	2700	50-3	1		40	6	← 50 Micrograms
30	900,000	29-43	0.4	3600	37-2		1	36	4	B_{12} given.
31	950,000	32-47	6.4	7700	38-2	3	1	50	6	
2-2-49	2.10	36-52	24.4	2600	26-7	1		61		
4	2.60	45-65	23.5	2800	30-6	2		57	5	
6	2.60	45-65	18.0	4360						
9	3.10	49-70	12.0	5200						
12	3.00	52-75		6200	55-1		2	42		
14	3.20	66-96		6400	62-2	1	1	30	4	
16	3.35	66-96		4900	59-1	1	1	34	4	
18	3.30	69-10								← 25 Micrograms
21	3.41	69-10		6000	60-2		1	35	2	B_{12} given
28	4.41	83-120		7550						

Figure 9.—*Case 6.* Satisfactory hematopoietic response to 50 micrograms of vitamin B_{12}.

Sixty days after treatment was started, erythrocytes numbered 4,410,000 per cu. mm. and the hemoglobin was 83 per cent of normal (12 gm. per 100 cc.).

CASE 7: The patient, a 48-year-old white female, was known to have had pernicious anemia for 15 years with sporadic treatment. When examined on December 16, 1948, she complained of numbness and tingling in the fingers and toes, a loss of sense of position, and difficulty with locomotion.

Examination of the nervous system showed the vibratory sense to be absent from both feet and knees; position sense was absent in both big toes, and the patellar and Achilles tendon reflexes were both exaggerated. They were recorded as 3 plus. Examination of the blood revealed a slight macrocytic anemia. Erythrocytes numbered 3,940,000 per cu. mm. and hemoglobin was 11.4 gm. per 100 cc. (80 per cent). She had histamine-fast achlorhydria. The patient was given intramuscular injections of 50 micrograms of vitamin B_{12} on December 16, 1948, and January 6 and January 31, 1949. Neurological examination on the latter date revealed that the vibratory sense had returned to both knees and both ankles. Position sense had also returned to both great toes. The patient volunteered the information that there had been definite improvement in the gait. The erythrocyte count had increased to 4,430,000 per cu. mm. of blood.

COMMENT ON THE CASES IN GROUP II

In this group of three patients attention is again called to the excellent hematopoietic response to injections of vitamin B_{12}. This is indicated by the increased number of reticulocytes appearing in the peripheral blood within 48 hours and attaining a peak response in 96 hours after treatment was started. In two patients the response was optimum. In one patient (Case 6) the response was submaximal wherein a secondary reticulocyte response appeared following the second injection of vitamin B_{12}.

The neurological symptoms complained of by these patients varied in degree. In one (Case 5) they were minor, and consisted of numbness and tingling of the fingers and toes, and inability to discern the vibrations of a tuning fork over the feet. These symptoms had disappeared entirely by the 15th day following the injection of vitamin B_{12}. In the other two patients, the neurological changes were advanced to a further degree. These consisted not only of paresthesias and loss of vibratory sense, but loss of position sense and disturbances of locomotor function as well. In one patient (Case 6), seven days after treatment was started the paresthesias were greatly diminished and the Romberg test became negative. The vibratory sense, however, remained impaired. In the third patient (Case 7) when examined six weeks after treatment was started, there was return of vibratory sense, return of position sense and pronounced improvement in locomotion.

GROUP III

One case of pernicious anemia complicated by pregnancy and sensitivity to liver extract.

CASE REPORT

CASE 8: The patient, a white female, aged 31, was first examined in May, 1947, when she was found to have macrocytic anemia accompanied by gastric achlorhydria. Erythrocytes numbered 3,200,000 per cu. mm. and hemoglobin was 12.5 gm. per 100 cc. The patient was given intramuscular injections of 40 units of liver extract once a week for six weeks. The reticulocytes attained a peak of 6 per cent and, at the end of this period erythrocytes numbered 4,360,000.

The general condition improved and the patient remained well on injections of 20 units of liver extract every ten days. On November 12, 1948, the patient reported that after each of the last four injections she had suffered considerable pain, redness and swelling at the site of injection, which subsided only after two or three days. The last injection was further complicated by nausea and a generalized urticarial rash. At this time the patient was five months pregnant. It was believed that she had become sensitive to liver extract, and it was necessary to discontinue this form of therapy. On January 6, 1949, erythrocytes had decreased to 3,120,000 per cu. mm. of blood, and hemoglobin to 10 gm. per 100 cc. At this time an intramuscular injection of 25 micrograms of vitamin B_{12} was given. Reticulocytes increased to 12 per cent and on January 20, 1949, erythrocytes numbered 4,340,000 per cu. mm.

COMMENT ON THE CASE IN GROUP III

Case 8 is clearly one of Addisonian pernicious anemia that had been adequately treated on liver extract until the course of the illness became complicated by pregnancy. Subsequently, the patient became sensitive to liver extract and further use of this medication was discouraged. There was an interim of several months when the patient could not be given liver therapy and during which time there was recurrence of anemia of slight degree. When vitamin B_{12} became available as a therapeutic agent for this patient, there was a satisfactory response of the blood following an injection of 25 micrograms. There were no signs of drug sensitivity, such as were exhibited when liver extract was given.

DISCUSSION

In the eight patients whose case histories have been reported here, satisfactory hematopoietic response occurred in all following an initial injection of 25 or 50 micrograms of vitamin B_{12}. An increase in reticulocytes became apparent in the peripheral blood usually within 48 hours and peak response was attained within 96 hours. In these patients the peak of the reticulocyte response ranged from as low as 9 per cent to as high as 32 per cent. In none of the patients were there the maximal reticulocytoses up to 40 per cent or more as indicated by Isaacs' formula (i.e., the greater the degree of anemia, the more pronounced should be the reticulocyte response). It is well known that such factors as

arteriosclerosis or low grade infection may modify reticulocyte response. However, the conversion of the bone marrow from one of the megaloblastic (rubriblastic) hyperplasia to one of normoblastic (metarubriblastic) distribution accompanied by a return of erythrocytes and hemoglobin to normal levels within 60 days indicates that satisfactory remission had been induced.

No better initial response is likely to be obtained in an uncomplicated case of Addisonian pernicious anemia by giving larger doses of vitamin B_{12} than those used in the cases reported. Since two of the patients, one having received 50 micrograms and the other 25 micrograms as initial injections, showed secondary reticulocyte response on second injections of similar amounts, it may be stated that some patients will require greater amounts than others to produce absolute optimal effects. Experience in the use of vitamin B_{12} in a larger group of patients will be required to help solve this problem.

Some investigators recommend that vitamin B_{12} should be given in certain dosage (e.g., 10 micrograms once or twice a week) in order to obtain satisfactory remission and maintain the blood cell count at normal levels. Information at hand suggests that vitamin B_{12} is the effective agent in liver extract and that its action is similar to that of liver extract. It may be well, therefore, to adopt criteria which have been applied to liver extract and give larger individual dose injections at longer intervals rather than smaller doses at frequent intervals. Clinical studies imply that the anti-pernicious anemia activity of one microgram of vitamin B_{12} is approximately equivalent to that of one U.S.P. unit of liver extract (Rickes). The intramuscular injection of 60 micrograms of vitamin B_{12} should be the minimal amount to effect a satisfactory reticulocyte rise and cause a return of the blood to normal in 60 days.

In preliminary work the authors have started out with initial doses of 50 micrograms but more recently have reduced this to 25 micrograms, to be repeated every two weeks until the blood has returned to normal. An injection of 30 to 50 micrograms at intervals of one month should be adequate to maintain normal erythrocyte and hemoglobin content in the blood.

Originally it was found that, in the treatment of Addisonian pernicious anemia with raw liver, the early manifestations of combined system disease were arrested or completely abated in due course of time. In the process of purification of liver extract, it was thought that this activity might be lost in the discarded side-fraction or might be totally destroyed. This was not entirely so. Although some of the factors may have been removed during the process of concentration, liver extracts on the whole have served well in ameliorating or stopping progression of the combined system disease. Unfortunately, this has not been the case with folic acid. Clinical experience has taught that this is not a safe drug to use in the treatment of pernicious anemia because it does not have a curative effect on the combined system disease or prevent its development.

With regard to vitamin B_{12}, our experience is in keeping with that of Castle[2] and Spies[12] that in the cases of early combined system disease there is prompt relief from the paresthesias arising from degeneration of the posterior columns. In patients with moderately advanced disease, there may be complete relief of paresthesias and amelioration or complete disappearance of symptoms arising from the lateral descending pyramidal tract.

At present under observation is a group of patients whose paresthesias, loss of vibratory sense and spasticity of gait have persisted in spite of large doses of liver extract. Recently, these patients have been given injections of vitamin B_{12}. Objectively, little change can be detected in their condition, but their spontaneous admissions are to the effect that they feel better and have noticed improvement in locomotion.

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